Dissecting a cellular RNA quality control system

Based at Case Western Reserve University, **Dr Kristian Baker's** research interests focus on RNA metabolism and how it is regulated by the cell. Coupling classic approaches with cutting-edge technologies, Dr Baker and her team have gained new insights into how the cell detects and eliminates faulty genetic intermediates. Known as RNA surveillance, this process protects the cell from the accumulation of defective proteins and acts as a quality control filter to both uphold fidelity and fine-tune gene expression.

The key to developing novel therapeutics for a large category of inherited diseases lies in a thorough understanding of the cellular pathways that respond to genetic mutations. Dr Kristian Baker, Associate Professor at Case Western Reserve University, is investigating an RNA quality control process that exists to recognise faulty genetic RNA intermediates in cells and prevent them from directing the production of non-functional proteins. Although in place to safeguard the cell, one downside of this vital activity is that it also contributes to the severity of genetic disorders caused by a certain class of gene mutation.

THE GENE EXPRESSION PIPELINE

Proteins are large, complex molecules that play essential roles in countless cellular processes. Critical for proper cell function, proteins act as catalysts for metabolic reactions, are involved in transmitting signals within and between cells, and offer essential structural and mechanical functions, amongst other roles. The protein building process starts at the DNA level. DNA encodes information in units referred to as genes, which individually contain the blueprint for each cellular protein. Gene information is copied onto a template called messenger ribonucleic acid, or mRNA, which is then read by cellular machines called ribosomes to produce a distinct protein product. Each protein is constructed from building blocks called amino acids which are placed in unique combinations dependent on the information encoded in the mRNA. It is imperative for the cell that this process – referred to as protein synthesis – occurs properly, and that the mRNA template is a faithful copy of the information encoded within the gene.

GUARDING FOR CORRECT PROTEIN FUNCTION

Mistakes introduced into an mRNA template can instruct for the production of non-functional or even toxic proteins that severely affect cellular performance and survival. To prevent the synthesis of such proteins, sophisticated surveillance systems are in place to recognise faulty mRNAs and target them for rapid removal from the cell. One of those systems is called nonsense-mediated mRNA decay (NMD). Its purpose is to find and eliminate mRNAs containing premature stop signals, which cause protein production to end too early and result in incomplete, truncated proteins. Such out-of-place stop signals do not encode for any amino acid and are referred to as nonsense mutations.

Nonsense-mediated mRNA decay (NMD) is essential for cell growth and development... Defects in NMD are linked with severe cognitive disabilities in humans

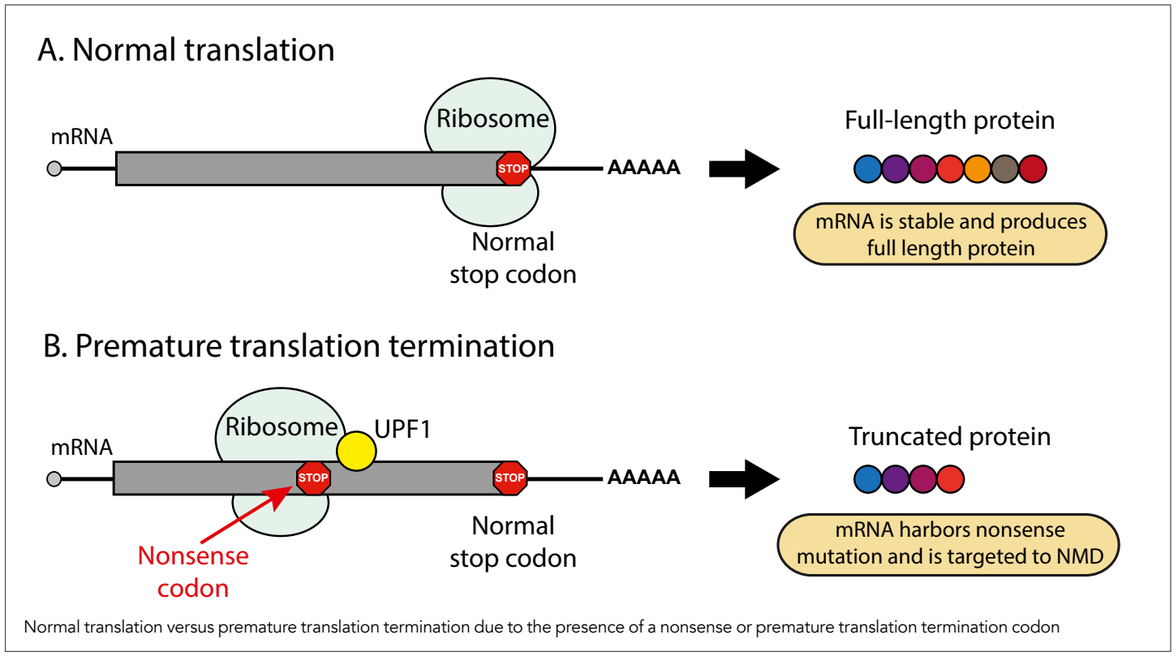
A DOWNSIDE TO EFFICIENT QUALITY CONTROL

Quality control is critical in all production processes, and in the cell, a functional nonsense-mediated mRNA decay pathway is essential for cell growth and development. Organisms with an inoperative pathway show developmental defects and cell death, and in humans, deficiencies in NMD are linked with severe cognitive disabilities.

Importantly, nonsense mutations are not necessarily only introduced into mRNA when information is copied from the gene, but can be present in the DNA template itself due to gene mutation. In fact, nonsense mutations represent between 20-30% of the genetic lesions identified for all types of inherited disorders. The problem lies in the scrutiny of the NMD surveillance process – in cases of gene mutation, all of the mRNA copied from that DNA harbours a nonsense signal and is targeted to NMD, effectively preventing production of any protein and leading to complete loss-of-gene function. For some nonsense mutations, however, their position within the gene is such that the incomplete protein produced – if sufficient levels of the mRNA existed – would have partial function. To combat genetic diseases that stem from nonsense mutations, it would thus be highly beneficial if NMD could be modulated, for instance by interfering with the recognition of the premature stop signal or by blocking the elimination of the mRNA. However, in order for the NMD pathway to be a precise and potent clinical target, the pathway and its functions in the cell need to be fully understood.

TIME FOR A STRATEGIC APPROACH

Dr Baker and her team at Case Western Reserve University focus their research around RNA metabolism with a special emphasis on nonsense-mediated mRNA decay. The Baker lab combines classic genetic and biochemical approaches with cutting-edge technologies to unravel important aspects of the mechanisms underlying NMD. In the process, they have also made discoveries that have furthered our ▸



Normal translation versus premature translation termination due to the presence of a nonsense or premature translation termination codon

overall understanding of gene structure and function.

A CLUE BEHIND TARGETED AND RAPID ELIMINATION OF FAULTY mRNAS

Major unresolved questions regarding the NMD pathway are how the system distinguishes between normal and nonsense-containing mRNAs, and what molecular events then occur to trigger the rapid degradation of the faulty mRNA. The Baker Lab recently revealed that the nonsense-mediated decay machinery interacts closely with ribosomes, the cellular machines that make proteins. Once the ribosome encounters a nonsense mutation in the mRNA, it halts, and a component of the NMD surveillance complex, UPF1, helps to release the ribosome from the defective mRNA. UPF1 mediates ribosome release by using energy from adenosine triphosphate (ATP) – the main energy storage molecule within cells. The precise timing of ribosome release is important for signalling rapid degradation of the mRNA; if cells harbour an inactive version of the UPF1 factor which is unable to use ATP, release of ribosomes from defective mRNAs is stalled and rapid degradation of the mRNA is prevented. This close interplay between the protein production and surveillance machineries represents a critical step in the clearance process and an exciting advance in our understanding of how NMD occurs in cells.

NEW DISCOVERIES ALONG THE ROAD

Ribonucleic acids (RNAs) are versatile molecules and their function is not limited to serving as templates for protein production. Notably, other RNA classes exist in cells and are known to play important roles during protein synthesis and in fine-tuning gene activity. Dr Baker and her team examined the influence of NMD on the metabolism and regulation of a set of these RNAs referred to as long non-coding RNAs (lncRNAs) and made some exciting observations: many lncRNAs were actively engaging with the protein production machinery and, at least from some, short protein chains were being produced. The biological relevance of these short protein chains has yet to be fully addressed. Nonetheless, the finding is provocative as it subverts prior ideas of an exclusively regulatory function of lncRNAs and indicates that so far, research may have missed a class of RNAs that direct the production of potentially important protein factors. In the same study, the Baker group revealed that a large fraction of lncRNAs are targeted by the nonsense-mediated decay machinery, further supporting the idea that some lncRNAs act as templates for protein synthesis. These observations, moreover, imply that NMD is important not only for surveillance of faulty mRNAs, but also in regulating cellular RNA levels and activity more broadly.

BLENDING SCIENCE AND EDUCATION

In addition to successfully pursuing her research agenda, Dr Baker is dedicated to providing a first-class training opportunity for junior researchers. Dr Baker's efforts are well known and attract top students eager to develop critical thinking and scientific method skills, and apply cutting-edge experimental approaches in their research. In her efforts to foster the future generation of scientific researchers, Dr Baker not only advises aspiring scientists from her own institution, but also hosts students from a local community college and high schools as well. Additionally, Dr Baker participates in a number of programmes that provide laboratory experience or professional development for women and under-represented groups in science, and has a reputation as a nurturing advisor and mentor.

Dr Baker's ground-breaking work on a critical cellular quality control system for mRNAs has revealed essential insights into its underlying mechanisms. Her research aims to unravel the complex workings of the process to illuminate how this pathway can be uniquely targeted for therapeutic purposes in the treatment of a vast array of human genetic disorders.

> **Combining classic genetic and biochemical approaches with cutting-edge technologies…the team has made exciting in-roads into our understanding of how faulty genetic intermediates are identified and eliminated by the cell**

Q&A

What has been the most memorable moment during your years in RNA research?

There is no one event that can be singled out – during my career, I have been extremely fortunate to train with outstanding mentors who have been generous with their time and knowledge, and work with talented young students whose curiosity and excitement make this job rewarding. The RNA research community has a reputation of collegiality and collaboration, and I have certainly benefitted personally and professionally from being a part of this wonderful international group of scientists.

Your recent findings demonstrate how the NMD and protein synthesis machineries (ribosomes) communicate – but how does the cell know this mRNA needs to be degraded?

This is the key question and a critical next step in our research. We anticipate that the energy released from ATP hydrolysis by UPF1 induces changes in the terminating ribosome that are sensed by the cellular RNA decay machinery so as to elicit rapid degradation of the mRNA. We are presently applying genetic, biochemical, and structural approaches to understand this change in ribosome function.

Are you planning to follow-up on your findings on protein fragments (peptides) derived from long non-coding RNAs?

Indeed, our finding that some lncRNAs direct the production of small proteins is unexpected, and determining a cellular function for these proteins is a major focus and exciting direction for my lab.

How realistic is the prospect of therapeutically targeting the NMD pathway to treat genetic disorders and what do you consider the biggest hurdles?

Clinical trials for the treatment of genetic diseases stemming from nonsense mutations are already underway. Existing treatments, however, were developed without a full comprehension of the mechanisms underlying recognition and rapid degradation of the mRNA expressed from these mutated genes. We foresee development of a second generation of therapeutics, based on a detailed understanding of the NMD process, that offer increased potency and greater safety for patients.

You are very active in mentoring young researchers – how do you benefit from this experience?

While I enjoy teaching in the classroom, my real passion is working alongside junior researchers, helping them develop their scientific method and laboratory techniques. Nurturing these skills certainly contributes to furthering our research goals, but even greater is the ability to share the excitement of scientific discovery with my students – many of whom are experiencing it for the very first time.

What are your long-term research goals?

Our first priority is to fully understand all aspects of how the cell executes NMD, and do so at a molecular resolution that will enable us to design new therapies in the treatment of genetic disorders stemming from nonsense mutations. On a grander scale, we will continue our efforts to learn how cells regulate RNA as a means to achieving gene expression patterns that give rise to the complex function of cells and organisms.

Detail

RESEARCH OBJECTIVES

Dr Baker's research explores how cells monitor RNA integrity, a process that is critical for maintaining proper gene expression and cell viability. Her ground-breaking work has enhanced our mechanistic understanding of RNA quality control and is creating opportunities for the development of advanced therapies to treat a broad class of inherited genetic diseases.

FUNDING

- National Institutes of Health (NIH-NIGMS)
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BIO

Completing her PhD at the University of British Columbia, Dr Kristian Baker joined the faculty at Case Western Reserve University in 2008. Currently an Associate Professor at the Center for RNA Science and Therapeutics, Dr Baker also serves as Chair of the Membership Committee for the international RNA Society, is a dedicated educator and mentor, and staunch advocate for women and under-represented groups in science.

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